- 2) providing a cell culture medium of a buffered, serumfree solution having a pH value from about 6.8 to about 7.6 comprising:
  - a) glucose;
- b) a biologically utilizable form of pantothenic acid or choline;
- c) at least one inorganic ion in a biologically utilizable form, wherein said ion is chloride ion, phosphate ion, calcium ion, magnesium ion, potassium ion, sodium ion or iron ion;
- d) L-buthionine-[S,R]-sulfoximine (BOS), wherein said BOS is present in a concentration of about 5  $\mu M$  to about 500  $\mu M$ ;
  - e) deionized water;
- f) a mitogen, wherein said mitogen stimulates said lymphocytes to grow; and
- g) optionally, at least one of a supplemental nutrient in a biological utilizable form wherein said supplemental nutrient is:
  - i) an L-amino acid;
  - ii) a vitamin; or
  - ii) at least one of pyruvate, adenine or inositol;
- 3) removing BSO from said cell culture medium thereby providing a BSO negative medium;
- 4) placing no more than half of said lymphocytes isolated from said individual of interest into BOS medium and into BOS negative medium;
- 5) placing no more than half of said lymphocytes isolated from said at least one control individual into BOS medium and into BOS negative medium, said media other than the media used in step 4);

- 6) determining growth responses of all of said lymphocytes in steps 4) and 5), said growth response measured by <sup>3</sup>H-thymidine incorporation of said lymphocytes;
- 7) expressing said growth response of said lymphocytes from said individual of interest as the ratio of lymphocyte growth in BOS medium to lymphocyte growth in BOS negative medium;
- 8) expressing said growth response of said lymphocytes from said control individuals as an average ratio of lymphocyte growth in BOS medium to lymphocyte growth in BOS negative medium; and
- 9) comparing said lymphocyte growth response from said individual of interest to the average growth response of said control individuals, wherein if the ratio of said lymphocyte growth response from said individual of interest to said average control is less than about 85%, said individual of interest has a glutathione deficiency.

Please amend claim 7 to read as follows:

- 7. A cell culture medium for testing the growth rate of lymphocytes, said medium comprising:
- a buffered, serum-free solution having a pH value from about 6.8 to about 7.6, said solution containing:
  - a biologically utilizable form of pantothenic acid or choline;
- at least one inorganic ions in a biologically utilizable form, wherein said ion is chloride ion, phosphate ion, calcium ion, magnesium ion, potassium ion, sodium ion, and iron ion;

cumene hydroperoxide, wherein said cumene hydroperoxide is present in a concentration of about 5  $\mu M$  to about 500  $\mu M$ ;

deionized water,

02/12/2003 13:06

a mitogen wherein said mitogen stimulates said lymphocytes to grow; and

optionally, at least one of a supplemental nutrient in a biological utilizable form wherein said supplemental nutrient is:

- a) an L-amino acid;
- b) a vitamin; or
- c) at least one of pyruvate, adenine or inositol.

Please amend claim 9 to read as follows:

9. The cell culture medium of claim 7, wherein said vitamins are selected from the group consisting of biotin, folinic acid nicotinamide, nicotinic acid, riboflavin, thiamin, vitamin B<sub>6</sub>, and vitamin B<sub>12</sub>.

Please amend claim 11 to read as follows:

11. The cell culture medium of claim 7, wherein at least one of said pyruvate, said adenine or said inositol supplements said cell culture medium at concentrations eliciting approximately a maximal response.

Please amend claim 12 to read as follows:

- 12. A method of determining if an individual of interest has a cysteine deficiency, comprising the steps of:
- 1) isolating lymphocytes from said individual of interest and from more than one control individual;

7137776

- 2) providing the cell culture medium of claim 7 (NAC medium);
- 3) removing NAC from said cell culture medium thereby providing a NAC negative medium;
- 4) placing no more than half of said lymphocytes isolated from said individual of interest into NAC medium and into NAC negative medium;
- from said at least one control individual into NAC medium and into NAC negative medium, said media other than the media used in step 4);
- 6) determining growth responses of all of said lymphocytes in steps 4) and 5), said growth response measured by <sup>3</sup>H-thymidine incorporation of said lymphocytes;
- 7) expressing said growth response of said lymphocytes from said individual of interest as the ratio of lymphocyte growth in NAC medium to lymphocyte growth in NAC negative medium;
- 8) expressing said growth response of said lymphocytes from said control individuals as an average ratio of lymphocyte growth in NAC medium to lymphocyte growth in NAC negative medium; and
- 9) comparing said lymphocyte growth response from said individual of interest to the average growth response of said control individuals, wherein if the ratio of said lymphocyte growth response from said individual of interest to said average control is greater than or equal to about 127%, said individual of interest has a glutathione deficiency.

Please add claim 13 to read as follows:

13. The method of claim 6, wherein said vitamin is selected from the group consisting of biotin, folinic acid nicotinamide, nicotinic acid, riboflavin, thiamin, vitamin B<sub>6</sub>, and vitamin B<sub>12</sub>.

Please add claim 14 to read as follows:

14. The method of claim 6, wherein said L-amino acid is selected from the group consisting of L-arginine, L-cysteine, L-glutamine, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-serine, L-threonine, L-tryptophan, L-tyrosine, and L-valine.

Please add claim 15 to read as follows:

15. The method of claim 6, wherein at least one of said pyruvate, said adenine or said inositol supplements said cell culture medium at concentrations eliciting approximately a maximal response.

## Please add claim 16 to read as follows:

16. The cell culture medium of claim 7, wherein said N-acetyl-L-cysteine is present in a concentration of about 150 mM.

Please add claim 17to read as follows:

17. The cell culture medium of claim 7, wherein said L-amino acid is selected from the group consisting of L-arginine, L-cysteine, L-glutamine, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-serine, L-threonine, L-tryptophan, L-tyrosine, and L-valine.

add B